Published in final edited form as:
J Hum Hypertens. 2009 September ; 23(9): 578–584. doi:10.1038/jhh.2008.168.

Hypertension genes and retinal vascular calibre: the Cardiovascular Health Study

C Sun[1], **JJ Wang**[1,2], **FM Islam**[1], **SR Heckbert**[3], **R Klein**[4], **DS Siscovick**[5], **BEK Klein**[4], and **TY Wong**[1,6]

[1]Centre for Eye Research Australia, University of Melbourne, Victoria, Australia

[2]Centre for Vision Research, University of Sydney, New South Wales, Australia

[3]Department of Epidemiology, University of Washington, Seattle, WA, USA

[4]Department of Ophthalmology & Visual Sciences, University of Wisconsin, Madison, WI, USA

[5]Departments of Medicine and Epidemiology, University of Washington, Seattle, WA, USA

[6]Singapore Eye Research Institute, National University of Singapore, Singapore

Abstract

We examined the associations of single nucleotide polymorphisms (SNPs) in three candidate hypertension genes, α-adducin (ADD1/G460W), β2-adrenergic receptor (ADRB2/Arg16Gly and Gln27Glu) and G-protein β3 subunit (GNB3/C825T), with retinal arteriolar calibre (an intermediate marker of chronic hypertension) and venular calibre. Data in 1842 participants (1554 whites and 288 African Americans) aged 69–96 years from the Cardiovascular Health Study with genotype and retinal vascular calibre data were included. A computer-assisted method was used to measure retinal vascular calibre. We analysed four SNPs and multilocus interaction for three genes. All SNPs were in Hardy–Weinberg equilibrium in whites and African Americans. The study had sufficient power to detect 0.5% of the total variance of retinal vascular calibre contributed by each SNP in the total population, except for the GNB3 gene variant. No significant associations between these SNPs in the genes studied and mean retinal arteriolar and venular calibre were found in single-gene or multilocus analysis (for example, age-, gender-, race-adjusted mean retinal arteriolar calibre was similar between participants who were ADD1/460W homozygotes and ADD1/G allele carriers, 166.2 vs 167.7 μm). In conclusion, this study found no evidence of an association of SNPs in candidate hypertension genes studied here with retinal vascular calibre.

Keywords

α-adducin gene; β2-adrenergic receptor gene; G-protein β3 subunit gene; single nucleotide polymorphisms; retinal arterioles; retinal venules

Introduction

Hypertension is a complex polygenic disease with both environmental and genetic risk factors conferring susceptibility to its pathogenesis.[1] Despite substantial progress, genes responsible for hypertension remain poorly understood.[2] Candidate–gene association studies, as used in

Correspondence: Dr TY Wong, Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, 32 Gisborne Street, East Melbourne, Victoria 3002, Australia. twong@unimelb.edu.au .

most genetic studies, have often failed to yield reproducible results.[3,4] This is partly due to the heterogeneous nature of the hypertension phenotype.[4]

One approach to reduce the heterogeneity is to use intermediate or subclinical phenotypes of hypertension. This has the advantage of allowing an analysis of a more specific and homogeneous definition of hypertension according to characteristics that are involved in the biological pathways linking candidate genes to blood pressure, potentially increasing the likelihood of gene discovery.[3]

Narrowed retinal arterioles are markers of hypertensive end-organ changes,[5] and retinal arteriolar calibre may also be an intermediate phenotype of hypertension and related cardiovascular diseases.[6,7] In this regard, studies show that retinal arteriolar calibre is heritable, [8] and narrowed calibre precedes the development of future hypertension in normotensive people.[9]

There has been considerable interest in three candidate genes for hypertension: α-adducin (ADD1), β2-adrenergic receptor (ADRB2) and G-protein β3 subunit (GNB3).10،11 In particular, four specific single nucleotide polymorphisms (SNPs) among these three genes have been linked to hypertension,1 left ventricular systolic function12 and to an intermediate phenotype of hypertension (for example, salt sensitivity). These SNPs are the G460W polymorphism in the ADD1 gene,13 the Arg16Gly and Gln27Glu polymorphisms in ADRB2 gene14 and the C825T polymorphism in the GNB3 gene.1،11 However, not all studies have consistently reported associations.[15]

In the (CHS), a population-based study of elderly whites and African Americans, we have previously demonstrated that smaller retinal arteriolar calibre is associated with elevated blood pressure[6] and larger venular calibre is associated with incident cardiovascular disease.7 We hypothesize that these three candidate genes for hypertension are associated with retinal vascular calibre and examined this in a large population-based study.

Materials and methods

Study population

We examined the association of four SNPs in three candidate genes for hypertension (ADD1, ADRB2 and GNB3) with retinal arteriolar and venular calibre in the CHS, a population-based study of in adults aged 65 years and more in the US.16 Details of the study population and methodology are described elsewhere.17 In brief, recruitment of the original cohort of 5888 persons took place at four field centres in the United States in 1989–1990 and 1992–1993. Of 4249 persons (95.5% of survivors) who were contacted at the 1997–1998 examination, we initially excluded 29 (0.7%) participants who did not self-identify as white or African American, 2236 (52.6%) people with missing data for retinal vascular calibre and 142 (7.7%) participants with no genotyping data, leaving 1842 (43.4% of the original 4249) participants for this analysis. Differences between those with and without retinal photographs or gradable photographs have been reported previously.6

Briefly, not having photographs or gradable photographs was associated with older age, female gender and African-American ethnicity. After controlling for age, gender and ethnicity, those excluded had a significantly higher mean diastolic blood pressure and fasting glucose levels, were more likely to have a history of coronary heart disease and stroke and to consume less alcohol than those included.

Retinal photography and grading

The retinal photography procedure has been described in detail elsewhere.6,[18] Briefly, during the 1997–1998 examination, two 45-degree non-mydriatic retinal photographs of one randomly selected eye of each participant were taken, one centred on the optic disc and the other on the macula (Early Treatment for Diabetic Retinopathy Study standard fields 1 and 2). Photographs were evaluated at the Fundus Photograph Reading Centre in Madison, Wisconsin, by two trained graders, masked to participant characteristics, according to a standardized protocol.[19]

To measure retinal vascular calibre, the fundus photographs were digitized with a high-resolution scanner. The diameters of all arterioles and venules coursing through a specified area half (1/2) to one (1) disc diameter from the optic disc were measured by a computer-assisted program.[18] The individual arteriolar and venular calibres were summarized as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) using formulas by Parr and Hubbard.19

The intra- and intergrader correlation coefficients ranged from 0.67 to 0.91 for retinal vascular calibre in CHS.

ADD1, ADRB2 and GNB3 genes genotyping

In CHS, genotyping of the ADRB2 gene was performed after polymerase chain reaction amplification on DNA extracted from blood samples collected at baseline examination using two methods.14 Gly16Arg and Gln27Glu variants were initially detected on a subset of 2166 participants using two restriction enzyme digestions. Genotyping was completed on the remainder of the cohort using a high-throughput TaqMan assay (Applied Biosystems, Foster City, CA, USA).[20] Both methods were used on a sample of 222 subjects representing all haplotypes with 99.6% agreement between methods. The ADD1/G460W and both ADRB2 SNPs were examined in the full CHS cohort and GNB3/C825T SNP for part of the cohort (all African Americans and one-third of the whites) using restriction-fragment-length polymorphism and/or a TaqMan allelic discrimination system on the basis of protocols described elsewhere.11,15

Definition of other variables

All participants underwent clinical and laboratory assessment of cardiovascular disease and its risk factors during the course of the study. Blood pressures were measured according to a standardized protocol and hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the combination of self-reported physician diagnosis of high blood pressure diagnosis and use of antihypertensive medications.21 Mean arterial blood pressure (MABP) was calculated as 2/3 of the diastolic blood pressure plus 1/3 of the systolic value. Diabetes was defined according to the American Diabetes Association Criteria (treatment with either oral hypoglycaemic agents and/or insulin in the year before the examination; or having fasting blood glucose of ≥126 mg per 100 ml for those not using any hypoglycaemic agents in the past year).[22] Body mass index (BMI) was calculated as kg m^{-2}. Blood collection and processing to determine fasting glucose and levels of serum lipids (total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and triglyceride) have been described previously.[23] Cigarette smoking and alcohol consumption status were ascertained from questionnaires. All variables defined were based on the 1997–1998 clinic examination, concurrent with retinal photography, except data on most blood chemistries (for example, HDL cholesterol and triglyceride used in this analysis), which was taken from the 1992–1993 examination.

Power calculation

Power calculations were performed using the Quanto program for continuous trait (http://hydra.usc.edu/gxe) according to the method described by Gauderman and Morrison.[24] The power of the main effect association study was computed for type 1 error a of 0.05 for dominant inheritance model. We determined that there were greater than 80% power to detect 0.5% of the total variance of retinal vascular calibre contributed by each SNP in the total population ($N = 1842$), except for the GNB3 gene variant (58% power in whites ($N = 468$) and even less power in African Americans). In addition, the study had limited power to detect gene–gene interactions and gene–environment effects in the total population.

Statistical analysis

Tests for Hardy–Weinberg equilibrium were analysed by the χ^2-test for both whites and African Americans. Where the reliability of *P*-values based on χ^2 approximations was questionable, *P*-values for Fisher's exact test were reported. Linkage disequilibrium (LD) and r^2 were computed for both whites and African Americans using the software program JLIN (a Java-based LD plotter).[25] We analysed the associations of each of the four SNPs with retinal vascular calibre. We defined the ADD1/460W allele and the GNB3/825T allele as the risk alleles for smaller arteriolar calibre and/or larger venular calibre. Both dominant and recessive genetic models were used based on previous reports.[11,14,26,27]

Initially, we performed analyses separately for each polymorphism in African Americans and whites. We then combined both races in the analysis with adjustment for race in a second analysis. As the direction of the associations across two races was similar, we only presented the combined population analysis with adjustment for race as our primary analysis (except for GNB3/T825C polymorphism for which the association directions differed significantly between whites and African Americans). We used ANCOVA (generalized linear model) to construct two models: model 1 adjusted for age, gender and ethnicity; model 2 additionally adjusted for fasting glucose, MABP, current smoking, BMI, total plasma cholesterol and HDL cholesterol if any significant association were found in model 1.

Potential interaction with age, gender, hypertension and cigarette smoking status were also examined. We performed additional stratified analysis when significant interaction was presented (for example, prespecified $P = 0.003$ based on Bonferroni correction for multiple testing of two traits (CRAE and CRVE) × 4 (SNPs) × 2 (gender)). All *P*-values are two sided and statistical analysis was carried out using SPSS version 12.0.1 (SPSS Inc., Chicago, IL, USA).

All SNPs were assessed in associations with retinal vascular calibre using a gene–gene interaction test with all combinations of two, three and four SNPs using the UNPHASED software.[28] The models were adjusted for age, gender and race.

Results

Both retinal arteriolar and venular calibre decreased with increasing of age (both *P* for trend < 0.001), and whites were more likely to have narrower retinal arteriolar calibre (*P* for trend = 0.01) and venular calibre (*P* for trend < 0.001) in the study population. There was no gender difference of different quartiles of retinal arteriolar or venular calibre (*P* for trend >0.05).

Genotype frequencies of the four SNPs were in Hardy–Weinberg equilibrium in both ethnic groups. ADRB2/Glu27 allele had high LD (= 1) and correlation ($r^2 = 0.69$ for whites and $r^2 = 0.17$ for African Americans) with Gly16 allele. The Gln27 allele had high LD with both the Gly16 and the Arg16 allele.

There was no significant relationship of the four SNPs of the ADD1, ADRB2 and GNB3 genes with either retinal arteriolar or venular calibre in whites, African Americans (model 1, data not shown) or in the total cohort after adjusting for age, gender and race, assessed using either dominant models or recessive models (Table 1).

We further tested SNP–SNP interaction and found no significant interactions for either CRAE or CRVE (Table 2). The interactions of age, gender, hypertension and cigarette smoking with any SNPs in relation to retinal vascular calibre were also not significant (all $P>0.003$).

Discussion

In this older adult population, we found no evidence of a single-marker association between three candidate genes of hypertension (ADD1, ADRB2 and GNB3) and retinal arteriolar or venular calibre.

The four common allelic variants of ADD1, ADRB2 and GNB3 genes have been hypothesized to play a key role in hypertension development, although studies have not consistently found association between these genes and blood pressure. ADD1 is a cytoskeletal protein and Na^+/K^+ exchange activity was found to be faster in carriers of ADD1/460W allele than in homozygotes for the 460G allele.[29] It has also been reported that compared with the wild-type variant, ADD1/460WW homozygotes have significantly reduced renal plasma flow, which is a potential intermediate phenotype of hypertension.[30] Stimulation of ADRB2, a predominant subtype of vascular adrenoceptor in the peripheral vascular smooth muscle, leads to relaxation of the vascular smooth muscle and thereby controls peripheral vascular resistance and blood pressure.[31] ADRB2 may also play a role in modulating coronary arteriolar tone.[32] Many studies have reported a significant association of the GNB3/825T allele with hypertension[1] and clinical stroke[33] in Caucasians. This may related to enhanced G-protein activation caused by enhanced signal transduction, proliferation and increased Na/H exchange.[34] Whether these genetic variants also have direct influence on the microcirculation and whether their associations with hypertension involve microvascular structural changes are unclear.

To our knowledge, there are no previous studies investigating these genes and retinal vascular calibre. A recent study reported that the ADD1/460W allele was associated with atherosclerosis, cardiovascular disease and stroke in a white population.[35] This is further supported by the finding that the ADD1/460W allele was found to be linked to an impaired endothelium-dependent vasodilation.[36] There is, however, no evidence of associations between genetic variation in the ADD1 and alterations in the microcirculation. Our data provide no evidence supporting a main effect of the ADD1 gene polymorphism on retinal vascular calibre.

In the current study, ADRB2 polymorphisms were not associated with retinal vascular calibre in either whites or African Americans. This is in good agreement with a recent report from the CHS showing ADRB2/Glu27 allele is not associated with blood pressure or subclinical atherosclerosis.[37] It suggests that factors other than blood pressure and related conditions may be involved in the relationship of ADRB2/Glu27 allele to lower risk of incident cardiovascular events compared to Gln27 homozygotes.[14]

A recent randomized clinical trial has shown the effect of GNB3 gene polymorphism on skin microcirculation via renin–angiotension system.[38] However, our data did not reveal an association of GNB3/C825T polymorphism with retinal vascular calibre in whites, which provide little support to the finding from a recent meta-analysis showing GNB3/825T allele carriers play a significant role in the pathogenesis of hypertension in Caucasians.[27] This may be due to lack of statistical power in the analysis.

Strengths of this analysis include the study of unrelated subjects from a population-based setting, the hypothesis-driven study with a biological understanding of the effect of genetic variants to increase statistical efficiency,3 and detailed information on a variety of environmental risk factors. Important limitations should also be mentioned. First, retinal photographs were taken 9 years after baseline study. As retinal vascular calibre has been demonstrated to be associated with cardiovascular mortality,[39] selective mortality might have obscured any finding of an association between retinal vascular changes and ADD1, ADRB2 and GNB3 gene polymorphisms (for example, those hypertensive participants carrying certain allele and with retinal vascular changes may have died before retinal photography). Second, a significant proportion of photographs were ungradeable in this elderly adult population due to the high prevalence of age-related cataract and other ocular pathologies. However, we believe that these ocular pathologies were randomly distributed regardless of retinal vascular calibre size. Third, findings from this older population may not be entirely applicable to populations of children and younger adults. It is likely that high prevalence of atherosclerosis and other cardiovascular risk factors may have masked the small to moderate effect of the associations of these candidate gene polymorphisms with retinal vascular calibre even when potential confounding effect has been taken into account. Finally, we studied only four SNPs, two of which were in marked LD. It has recently been found that the effects of ADD1 and GNB gene polymorphisms explained less than 1% of the variance in blood pressure traits.[40] It is unlikely that a single gene will explain a large proportion of the variance in retinal vascular calibre. Multiple alleles are likely to be involved in the regulation of complex traits. Many unmeasured genes, each with a small to moderate effect, remain to be discovered for common diseases such as hypertension, and for retinal vascular calibre variations.

In summary, in this large population-based study, we found no association of three candidate genes for hypertension (ADD1, ADRB2 and GNB3) with either retinal arteriolar or venular calibre. These data provide little evidence that these gene polymorphisms play a significant role in determining retinal vascular calibre.

What is known about this topic

- Three candidate genes for hypertension (single nucleotide polymorphisms of α-adducin (ADD1/G460W), β2-adrenergic receptor (ADRB2/Arg16Gly and Gln27Glu) and G-protein β3 subunit (GNB3/C825 T) genes) have been previously found to be associated with hypertension and cardiovascular diseases.[1, 10–15]
- Retinal vascular calibre is determined genetically, and narrowed retinal arteriolar calibre is an intermediate marker of hypertension and may contribute to individual susceptibility to the development of hypertension.[8,9]

What this study adds

- This study demonstrates that polymorphisms of ADD1, ADRB2 and GNB3 genes are not associated with either retinal arteriolar or venular calibre.

Acknowledgments

The research reported in this paper was supported by contracts N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133 and U01 HL080295 from the National Heart, Lung, and Blood Institute, and grant AG015366 from National Institute on Aging, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided by grant R21-HL077166 from NHLBI, NIH and the Sylvia and Charles Viertel Clinical Investigator Award (TYW). A full list of principal CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm.

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Table 1

Associations of ADD1, ADRB2 and GNB3 allele with retinal vascular caliber

Genotype	N (%)	*CRAE* *Mean (95% CI)*[a]	*CRVE* *Mean (95% CI)*[a]
ADD1			
GG+WG	1792 (96.3)	166.2 (165.0, 167.5)	193.7 (192.6, 194.8)
460W homozygotes	49 (2.7)	167.7 (162.2, 173.2)	197.6 (192.5, 202.6)
P-value		0.60	0.13
ADRB2			
Arg/Arg+Arg/Gly	1180 (64.2)	165.8 (164.4, 167.1)	193.8 (192.5, 195.0)
Gly16 homozygotes	658 (35.8)	167.3 (165.5, 169.0)	193.7 (192.1, 195.3)
P-value		0.10	0.92
ADRB2			
Glu27 homozygotes	292 (15.6)	165.8 (163.3, 168.3)	192.0 (189.7, 194.3)
GluGln+GlnGln	1548 (84.4)	166.3 (165.1, 167.6)	194.0 (192.8, 195.1)
P-value		0.68	0.08
GNB3[b]			
TT+TC	248 (53)	165.0 (162.7, 167.3)	189.4 (187.3, 191.6)
825C homozygotes	220 (47)	166.0 (163.6, 168.5)	190.2 (187.9, 192.5)
P-value		0.53	0.63

Abbreviations: ADD1, α-adducin; ADRB2, β2-adrenergic receptor; CI, confidence interval; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; GNB3, G-protein β3 subunit.

[a] Adjusted for age, gender and race.

[b] In whites only.

Table 2

Interaction tests between the SNPs studied, and between the SNPs and risk factors, in relation to retinal vascular caliber

Risk factors	***Test results for SNPs and risk factors,* P-*value***							
	CRAE				***CRVE***			
	ADD1 G460W	***ADRB2 Arg16Gly***	***ADRB2 Gln27Glu***	***GNB3 C825T***	***ADD1 G460W***	***ADRB2 Arg16Gly***	***ADRB2 Gln27Glu***	***GNB3 C825T***
Age	0.93	0.01	0.89	0.68	0.32	0.87	0.77	0.28
Gender	0.07	0.92	0.64	0.88	0.09	0.76	0.65	0.72
Hypertension	0.18	0.39	0.86	0.44	0.18	0.29	0.17	0.50
Smoking	0.03	0.62	0.94	0.13	0.05	0.54	0.91	0.31
SNPs	*Test results for SNP–SNP interactions,* P-*value*							
ADD1/G460W	—	0.25	0.33	0.71	—	0.36	0.02	0.76
ADRB2/Arg16Gly	—	—	0.36	0.97	—	—	0.04	0.97
ADRB2/Gln27Glu	—	—	—	0.98	—	—	—	0.70
GNB3/C825T	—	—	—	—	—	—	—	—

Abbreviations: ADD1, α-adducin; ADRB2, β2-adrenergic receptor; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; GNB3, G-protein β3 subunit; SNP, single nucleotide polymorphism.